# Pollen Viability and Germination in Some Orchid Hybrids

*by*

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## Introduction

Singapore has become an important orchid breeding center producing many well-known hybrids of international acclaim. Such successful results are achieved by the continued efforts of scientists at Botanic Gardens and local horticulturists over a period of three decades. In Singapore the first hybrid that appeared was *Vanda Miss Joaquim* in 1893 (Holttum, 1953). Nevertheless, our knowledge about the cytogenetics and pollen physiology of local orchid hybrids is very limited. The importance of such studies need not be over-emphasized since pollen viability and germination form the essential requirements to produce good and the required hybrids. Not every cross pollination will bear fruit and it is the common experience of every orchid breeder that more than 60 per cent of the pollinations made would end up with no fruits or fruits without seeds. One of the main reasons for such failures could be the pollen sterility and some of these problems are under study (Rao and Goh, 1970).

Very few studies are made on orchid pollen germination. Molisch determined the optimal sucrose concentration for 6 European species (Withner, 1959). Miwa (1937) germinated the pollen of certain orchid species and their hybrids to test the longevity and viability of stored orchid pollen. Curtis and Duncan (1947) studied 11 species in 7 genera. The present paper outlines the morphology of pollinia, pollen structure, viability and germination of some well-known local hydrids.

## Materials and Methods

Pollinia of the following hybrids were collected from Mandai Gardens now known as Singapore Orchids Pte. Ltd.: (i) *Arachnis Maggie Oei* var. *Red Ribbon,* (ii) *Arachnis Maggie Oei* var. *Yellow Ribbon,* (iii) *Aranda Hilda Galistan,* (iv) *A. Lucy Laycock* (long spray), (v) *A. Lucy Laycock* (short spray), (vi) *A. Wendy Scott* var. *Greenfield,* (vii) *Aranthera Lilliput,* (viii) *Vanda Poepoe* and (xi) *Vanda Josephine* (from the garden at Botany Department, University of Singapore).

Fresh pollinia from open flowers were collected between 2-3 p.m. and used within 1 hr. after collection, using half a pollinium in each case.

The following culture media were used: (*a*) distilled water as the control medium (*b*) stigmatic extract — the appropriate number of fresh stigmas were ground in 10 mls. of distilled water and the clear filtrate of the mixture was then used for germination. The number of stigmas used in such preparation varied from

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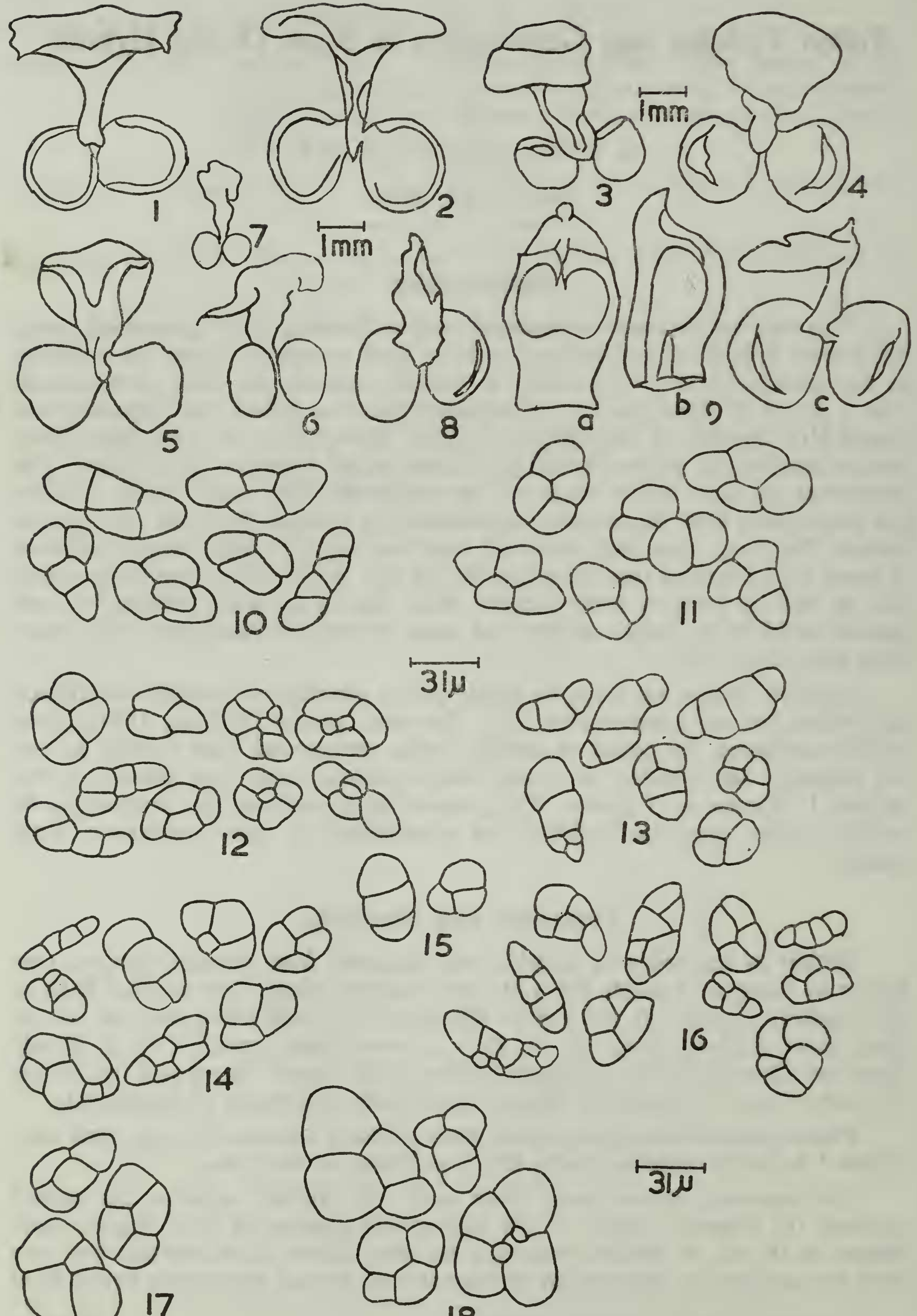
* Currently at Botanic Gardens, Singapore.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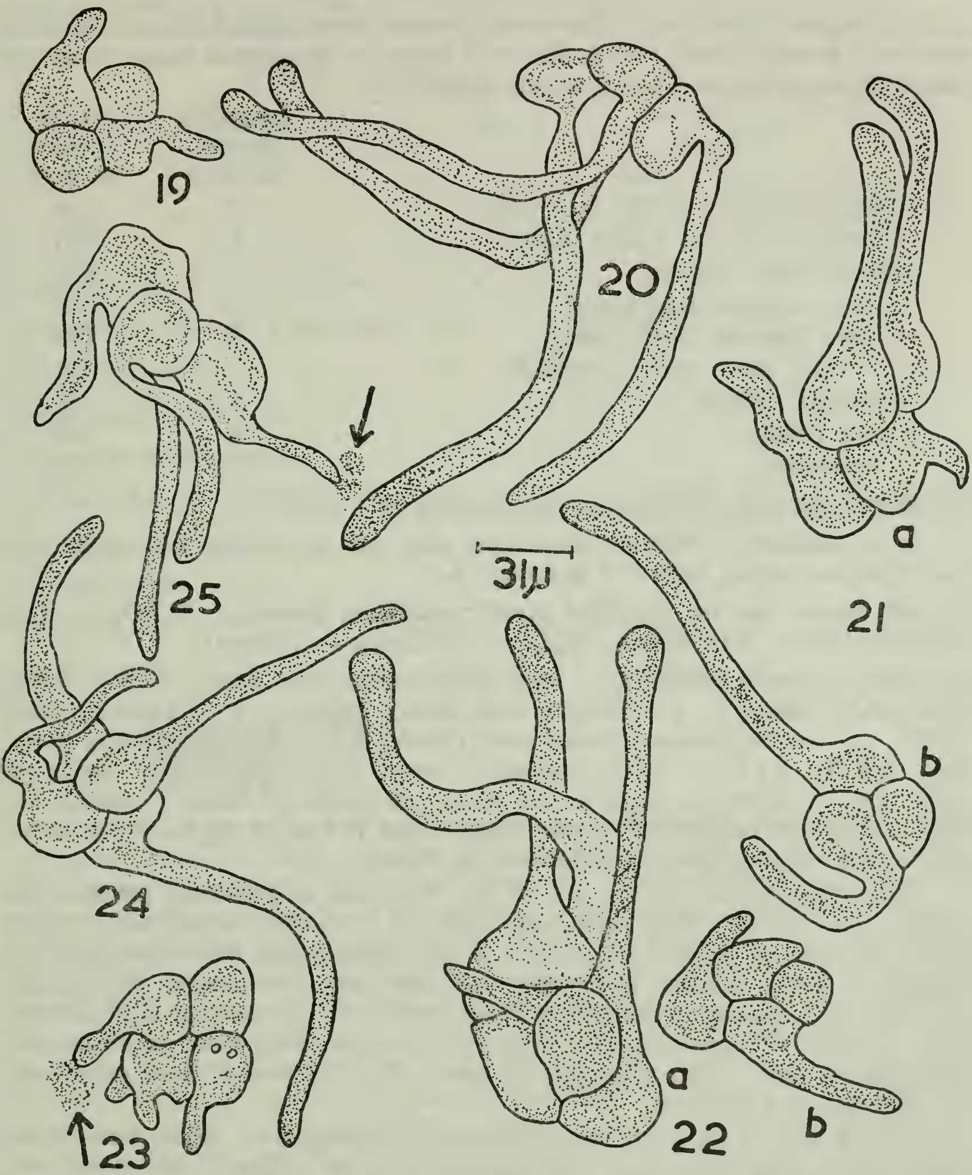


Figures 19–25: Pollen germination in *Vanda Josephine*. Fig. 19: in dist. water, note the short tubes. Fig. 20: in stigmatic medium at 2 stigmas/10 ml of dist. water; all 4 grains in the tetrad germinated. Fig. 21: in 4% sucrose a & b different sized pollen tubes with tapering ends. Fig. 22: in salt-boron medium, a — pollen tubes typical match-stick shaped, some with vacuoles; b — short tube, with broad ends. Fig. 23: in GA ($10^{-7}$ mg/L) medium short pollen tubes with exudates (arrow). Fig. 24: in borax medium, with all the grains in tetrad germinated. Fig. 25: in kinetin ($10^{-6}$mg/L) medium with pollen exudate (arrow).

*See opposite page*

Figures 1–18. Figs. 1–9: Pollinia of different orchid hybrids. Figs. 1 & 2: *Arachnis Maggie Oei* var *Red Ribbon* and var. *Yellow Ribbon* respectively. Figs. 3–6: *Aranda Hilda Galistan; Lucy Laycock* (long spray); *Lucy Laycock* (short spray); *Wendy Scott* var. *Greenfield* respectively. Fig. 7: *Aranthera Lilliput*. Fig. 8: *Vanda Poepoe*. Fig. 9: *Vanda Josephine*. a-column, b-$\frac{1}{2}$ stigma, c-pollinia. Figs. 10–18: Tetrads showing microspore arrangement and supernumerary spores. Fig. 10: *Arachnis Maggie Oei* var *Red Ribbon*. Fig. 11: *A. Maggie Oei* var. *Yellow Ribbon*. Fig. 12: *Aranda Hilda Galistan*. Fig. 13: *A. Lucy Laycock* (long spray). Fig. 14: *A Lucy Laycock* (short spray). Fig. 15: *A. Wendy Scott* var. *Greenfield*. Fig 16: *Aranthera Lilliput*, Fig. 17: *Vanda Poepoe*. Fig. 18: *Vanda Josephine*.

½, 1, 2, 3 stigmas/10 mls. of distilled water. In some cases all the four concentrations were used, in others only 1 or 2. The total length of the stigmas ranged from 3-8 mm in different hybrids. Their sizes and weights are:

| *Plants* | *Length of stigma (in mm)* | *Wt. of 1 stigma (in mg)* |
|---|---|---|
| *Arachnis Maggie Oei* var. *Red Ribbon* | 8 | 198 |
| *A. Maggie Oei* var. *Yellow Ribbon* | 8 | 180 |
| *Aranda Hilda Galistan* | 5 | 101 |
| *A. Lucy Laycock* (long spray) | 5 | 96 |
| *A. Lucy Laycock* (short spray) | 5 | 109 |
| *A. Wendy Scott* var. *Greenfield* | 6 | 87 |
| *Aranthera Lilliput* | 3 | 16 |
| *Vanda Poepoe* | 8 | 84 |
| *V. Josephine* | 8 | 85 |

(*c*) Sucrose medium with varying concentration of 1–10%.

(*d*) Borax medium — Sodium tetraborate was used to prepare the media with concentration ranging from $10^{-8}$ to 10 mg./L.

(*e*) Salt boron medium (modified Knop's medium). Formula: $H_3BO_3$ — 100, $Ca(NO_3)_2.H_2O$ — 300, $MgSO_4$. $7H_2O$ — 200, $KNO_3$ — 100mg/L.

(*f*) Different growth substances — well known auxins like: Indole — 3 — Acetic Acid (IAA), Indole — 2 — Butyric Acid (IBA), Indole — 3 — Propionic Acid (IPA), $\alpha$ — Naphthalene Acetic Acid (NAA), 2,4 — Dichlorophenoxy acetic acid (2,4–D), 2,4,5 — Trichlorophenoxy acetic acid (2,4,5–TPA), source of gibberellin — Gibberellic Acid K salt (GA), and source of Kinin — Kinetin (K) were used in the concentrations that ranged from $10^{-8}$ to 10 mg/L. Either all or only certain concentrations were found to be effective.

Solid watch glasses (4 x 4 x 1.75 cm) were used to germinate pollinia. The pollinia were cut into small pieces and dispersed in 1 ml of the medium. A glass cover was used to prevent evaporation of the medium and the watch glass was placed in a humid chamber to prevent evaporation and condensation. Diffuse light of 235 F. C. S. (during day-time) and temperature of 28°C prevailed in the laboratory. Duration of experiment was limited to 24 hours since most pollen did not germinate or their pollen tubes did not grow after this period. Each experiment was repeated at least twice.

At the end of 24 hours, the percentage germination of pollen tetrads and pollen cells, pollen tube length and morphology were studied. Lactophenol with cotton blue was used for staining. At least 225 tetrads were studied for each treatment to assess the percentage germination and 90 tubes for tube length determination.

## Observations and Results

Pollinia and pollen grains: In orchids the pollen tetrads develop into mature structures called compound pollen grains and these are united to form pollinia. In all of them two pollinia are present per flower (Figs. 1–9). The pollinia of different hybrids were excised for comparative study. In size the *Aranthera* pollinium was smallest among the 9 hybrids studied (Fig. 7). Pollinia of *Aranda* hybrids were smaller than those of *Arachnis* or *Vanda* (Figs. 1–6, 8, 9). The disc was flat in *Arachnis*, triangular in *Aranda*, and of irregular shape in *Vanda*. Even among the

Table 1. Morphology of the pollen tetrads, size and arrangement of pollen cells in them.

| Names of Orchids | Morphology of Pollen Tetrads | | | | | |
|---|---|---|---|---|---|---|
| | Size, μ | | Arrangement of grains in tetrads in % | | | |
| | L × B | L/B | isobilateral | tetrahedral | T-shaped | linear |
| *Arachnis Maggie Oei var. Red Ribbon* | 37 × 23 | 1.61 | 92 | 6 | 1 | 1 |
| *Arachnis Maggie Oei var. Yellow Ribbon* | 40 × 27 | 1.48 | 90 | 8 | 2 | — |
| *Aranda Hilda Galistan* | 33 × 24 | 1.37 | 82 | 16 | — | 2 |
| *A. Lucy Laycock* (long spray) | 34 × 24 | 1.42 | 72 | 22 | 2 | 4 |
| *A. Lucy Laycock* (short spray) | 32 × 25 | 1.28 | 88 | 8 | 2 | 2 |
| *A. Wendy Scott var. Greenfield* | 38 × 22 | 1.73 | Most of them are supernumerary spores. Normal tetrad — only about 1% | | | |
| *Aranthera Lilliput* | 28 × 20 | 1.40 | 89 | 9 | 1 | 1 |
| *Vanda Poepoe* | 42 × 27 | 1.56 | 68 | 32 | 1 | 1 |
| *Vanda Josephine* | 38 × 27 | 1.41 | 72 | 37 | 1 | — |

L — Length of pollen tetrad
B — Breadth of pollen tetrad
L/B — Length/breadth ratio.

*Aranda* hybrids the pollinia of each hybrid were distinctly different from the others in having disks and stalks of different shape and size (Figs. 3–6). The pollinia were teased to separate the tetrads. These are diagrammatically represented in Figs. 10–18. Each figure shows the variation in size, shape, arrangement as well as the number of cells in each tetrad or the unit (is one which had more than 4 cells). In addition, the cells of the tetrad or unit also varied in size. Some of them e.g. *Vanda Miss Joaquim, Vanda Poepoe, Aranthera Lilliput* had one small and three big grains or vice-versa (Figs. 14, 16–18, 22, 28, 29). Two big and two small grains were also common in some (Figs. 13, 22). Supernumerary spores were also common in many (Figs. 12–14, 16, 18) and the individual unit had 5, 6 or 8 cells. In such units, which had supernumerary spores, at least two of them were big and of comparable size with the spores of the majority of the tetrads and the remaining were generally small (Figs. 12, 13, 18). Pollen tetrads show isobilateral, tetrahedral, linear and 'T' shaped arrangement. The percentage occurrence of each type is recorded in Table 1. Comparatively, the two latter conditions were rare and the isobilateral type was most common. The average size of pollen tetrad ranged from 28 x 28 μ to 42 x 27 μ. The linear tetrads were much smaller in size when compared with the others (Figs. 12, 14, 16).

Pollen germination: Positive responses resulted in the pollen germination of *Vanda* hybrids and these details will be considered first, followed by the results of other hybrids.

(*a*) Distilled water (control medium). In *V. Poepoe* and *V. Miss Joaquim* 27 and 30% of the tetrads germinated with an average tube length of 15 and 19 μ respectively (Tables 2, 3; Figs. 19, 26, 30, 31).

Table 2. *Vanda Poepoe*. Percentage germination and average pollen tube length in different media after 24 hrs. at room temperature.

| Media | Concentrations | No. of grains germinated per tetrad, in % | | | | %G | $\mu$ L |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | |
| Distilled water (control) | | 18 | 5 | 2 | 2 | 27 | 15 |
| Stigmatic extract | 1 stigma /10 mls. distilled $H_2O$ | 20 | 21 | 14 | 10 | 65 | 57 |
| | 2 stigmas /10 mls. distilled $H_2O$ | 27 | 30 | 13 | 13 | 83 | 68 |
| | 3 stigmas /10 mls. distilled $H_2O$ | 21 | 20 | 8 | 4 | 53 | 44 |
| Sucrose | 2% | 28 | 20 | 4 | 12 | 64 | 56 |
| | 4% | 23 | 25 | 24 | 13 | 85 | 75 |
| | 6% | 8 | 16 | 24 | 41 | 89 | 83 |
| | 8% | 15 | 11 | 12 | 12 | 50 | 48 |
| | 10% | 11 | 9 | 11 | 8 | 49 | 40 |
| Borax | $1 \times 10^{-5}$ mg. /L | 26 | 6 | 2 | 0 | 34 | 18 |
| | $1 \times 10^{-4}$ mg. /L | 20 | 12 | 2 | 2 | 36 | 21 |
| | $1 \times 10^{-3}$ mg. /L | 27 | 10 | 0 | 2 | 39 | 36 |
| | $1 \times 10^{-2}$ mg. /L | 20 | 14 | 4 | 2 | 40 | 36 |
| | $1 \times 10^{-1}$ mg. /L | 25 | 12 | 8 | 2 | 47 | 38 |
| | $1 \times 10—^{0}$ mg. /L | 24 | 16 | 8 | 13 | 50 | 55 |
| | $1 \times 10—^{1}$ mg. /L | 16 | 14 | 8 | 2 | 40 | 37 |
| Salt-boron medium | | 22 | 32 | 10 | 8 | 72 | 72 |

% G — Perc. germination

$\mu$ L — Avg. pollen tube length in $\mu$

Table 3. *Vanda Poepoe*. Percentage germination and average pollen tube length in optimal concentrations of different media, after 24 hrs. at room temperature.

| Media | Optimal Concentrations | No. of grains germinated per tetrad, in % | | | | %G | μ L |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | |
| Distilled water (control) | | 18 | 5 | 2 | 2 | 27 | 15 |
| IAA | 1 × $10^{-6}$ mg./L | 27 | 24 | 14 | 6 | 71 | 71 |
| IBA | 1 × $10^{-5}$ mg./L | 21 | 25 | 15 | 9 | 70 | 66 |
| IPA | 1 × $10^{-6}$ mg./L | 24 | 17 | 11 | 3 | 55 | 41 |
| NAA | 1 × $10^{-4}$ mg./L | 27 | 26 | 20 | 5 | 78 | 82 |
| 2,4-D | 1 × $10^{-6}$ mg./L | 31 | 21 | 15 | 0 | 67 | 60 |
| 2,4,5-TPA | 1 × $10^{-6}$ mg./L | 27 | 25 | 6 | 7 | 65 | 40 |
| GA | 1 × $10^{-5}$ mg./L | 26 | 23 | 5 | 7 | 61 | 75 |
| Kinetin | 1 × $10^{-5}$ mg./L | 24 | 15 | 9 | 7 | 55 | 34 |

% G — Perc. germination

μ L — Avg. pollen tube length in μ

(*b*) Stigmatic extract medium: For both the hybrids the optimal concentration of stigmatic extract was 2 stigmas/10 ml. of distilled water. The percentage germination for *V. Poepoe* and *V. Miss Joaquim* was 83% and 79% with an average tube length of 68 and 87 μ respectively and these results were better than in the control medium (Tables 2, 3; Figs. 20, 27, 33). The response at other concentrations was variable in terms of percentage germination.

(*c*) Sucrose medium: For *V. Poepoe* the optimal concentration of sucrose was 6% and 89% of the tetrads germinated with an average tube length of 83 μ (Fig. 28). In *V. Miss Joaquim* 92% of the tetrads germinated in 4% sucrose medium (optimal concentration) with an average tube length of 74 μ (Fig. 21a, b). Other variations observed in media with higher or lower concentrations are given in Tables 2, 3 and in all the concentrations used the response was better than in the control medium.

(*d*) Borax medium: In media with different concentrations of borax better results than control were obtained in *V. Poepoe*. The optimal concentration for the two hybrids were 1 and 0.1 mg/L respectively (Figs. 24, 34) and the response of *V. Miss Joaquim* was much better than *V. Poepoe* (Tables 2, 3).

(*e*) Salt-boron medium: When compared with control or borax medium the values obtained in this medium were better in the case of *V. Poepoe* (Table 2). In case of *V. Miss Joaquim* the percentage germination was almost like that of control but tube length improved. These results were poor when compared with those obtained in optimal concentration of borax medium (Table 3, Fig. 22a, b).

Table 4. *Vanda Josephine.* Percentage germination and pollen tube length in different media after 24 hrs. at room temperature.

| Media | Concentration | No. of grains germinated per tetrad, in % | | | | %G | μ L |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | |
| Distilled $H_2O$ (control) | | 29 | 5 | 2 | 0 | 36 | 19 |
| Stigmatic extract | $\frac{1}{2}$ stigma /10mls. distilled $H_2O$ | 21 | 11 | 2 | 4 | 39 | 55 |
| | 1 stigma /10mls. distilled $H_2O$ | 12 | 9 | 6 | 21 | 48 | 77 |
| | 2 stigmas /10mls. distilled $H_2O$ | 15 | 6 | 20 | 28 | 79 | 87 |
| | 3 stigmas /10mls. distilled $H_2O$ | 7 | 19 | 21 | 2 | 39 | 47 |
| Sucrose | 2% | 8 | 15 | 31 | 21 | 75 | 62 |
| | 4% | 5 | 8 | 22 | 57 | 92 | 74 |
| | 6% | 9 | 21 | 14 | 36 | 80 | 37 |
| | 8% | 16 | 1 | 2 | 1 | 20 | 21 |
| | 10% | 11 | 0 | 0 | 0 | 11 | 14 |
| Borax | $1 \times 10^{-3}$ mg. /L | 28 | 17 | 0 | 1 | 46 | 27 |
| | $1 \times 10^{-2}$ mg. /L | 44 | 8 | 8 | 0 | 60 | 41 |
| | $1 \times 10^{-1}$ mg. /L | 24 | 19 | 24 | 18 | 85 | 84 |
| | $1 \times 10^{0}$ mg. /L | 21 | 13 | 6 | 3 | 43 | 63 |
| | $1 \times 10$ mg. /L | 9 | 2 | 1 | 1 | 13 | 19 |
| Salt-boron medium | | 26 | 6 | 5 | 1 | 38 | 32 |

% G — Perc. germination

μ L — Avg. pollen tube length in μ

(*f*) Media with different growth substances: Cultures in NAA ($10^{-4}$ mg/L) showed the highest percentage germination than in other auxin media, attaining 74% and the longest tube length of 82 $\mu$. Effect of IBA was similar of that of IAA giving better results than 2,4–D and 2,4,5–TPA media (Table 4). In case of *V. Poepoe* for IAA, IPA, 2,4–D and 2,4,5–TPA the optimal concentration was $10^{-6}$ mg/L. Of these four auxin media the highest percentage (71%) germination and the longest tubes (71 $\mu$) were formed in IAA medium and the lowest percentage germination was obtained in IPA medium (55%); the shortest tubes were produced in 2,4,5–TPA medium (40 $\mu$).

Table 5. *Vanda Josephine*. Percentage germination and pollen tube length in optimal concentration of different media after 24 hrs. at room temperature.

| Media | Optimal Concentrations | No. of grains germinated per tetrad, in % | | | | %G | $\mu$ L |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | |
| Distilled water (control) | | 29 | 5 | 2 | 0 | 36 | 19 |
| IAA | $1 \times 10^{-5}$ mg. /L | 33 | 12 | 12 | 7 | 64 | 43 |
| IBA | $1 \times 10^{-5}$ mg. /L | 32 | 12 | 6 | 2 | 52 | 35 |
| IPA | $1 \times 10^{-5}$ mg. /L | 20 | 15 | 11 | 3 | 49 | 59 |
| NAA | $1 \times 10^{-5}$ mg. /L | 22 | 14 | 12 | 3 | 51 | 47 |
| 2,4-D | $1 \times 10^{-5}$ mg. /L | 19 | 18 | 11 | 3 | 51 | 51 |
| 2,4,5-TPA | $1 \times 10^{-5}$ mg. /L | 33 | 18 | 6 | 3 | 60 | 45 |
| GA | $1 \times 10^{-6}$ mg. /L | 29 | 16 | 5 | 1 | 51 | 39 |
| Kinetin | $1 \times 10^{-6}$ mg. /L | 14 | 15 | 18 | 17 | 64 | 64 |

% G — Perc. germination
$\mu$ L — Avg. pollen tube length in $\mu$

The optimal concentration for GA and K was $10^{-5}$ mg/L (GA), resulting in 61% germination and 75 $\mu$ tube length and 55% germination and 34 $\mu$ tube length respectively (Table 4). The different auxin, gibberellin and kinetin media, at various optimal concentration, gave better results than the control medium. When compared with borax medium, poorer results were obtained in the two auxin media, i.e. IPA or 2,4,5–TPA. In all the others, including GA medium, better results were recorded. Kinetin medium was less favourable than the borax medium and similarly, gibberellin and kinetin media were not so favourable when compared with stigmatic extract or sucrose media (Tables 2, 3).

In case of *V. Miss Joaquim* germination and elongation of pollen tubes increased appreciably in auxins, GA and K media, when compared with the control or salt-boron medium. The optimal concentration was between $10^{-5}$ and $10^{-6}$mg/L. Higher concentrations were inhibitory for germination and tube growth. This was true in all the media except in case of IAA where even at 0.1 mg/L the percentage germination and tube length were very similar to that of the control (Table 5). In IAA and kinetin media better germination resulted when compared with the other auxins or GA media. The media with auxins, GA or K (at optimal concentrations) were not so favourable as stigmatic extract, sucrose or borax media (Tables 4, 5).

*Hybrids other than Vanda*

The pollen germination studies in case of: (*a*) *Arachnis Maggie Oei* var. *Red Ribbon,* (*b*) *Arachnis Maggie Oei* var. *Yellow Ribbon,* (*c*) *Aranda Hilda Gallistan,* (*d*) *Aranda Lucy Laycock* (long spray), (*e*) *Aranda Lucy Laycock* (short spray), (*f*) *Aranda Wendy Scott* var. *Greenfield* and (*g*) *Aranthera Lilliput,* gave very poor results in the different media used. In most of them no germination was seen with the exception of *Arachnis* Maggie Oei var. *Yellow Ribbon* the pollen of which showed small protrusions in the control medium. In borax medium there was good germination and pollen tube growth at two lower concentrations i.e. $10^{-5}$ (25% G, 11 $\mu$) and $10^{-4}$ (61% G, 32 $\mu$) mg/L. In salt boron medium 52% germination and 29 $\mu$ tube length resulted. In other media (stigmatic extract with 1, 2 or 3 stigma/10 ml of $H_2O$; sucrose 2-10%) there was no germination. Likewise, the different hybrids did not germinate in the auxin, GA or K media at various concentrations mentioned previously.

*Morphology of pollen tubes:*

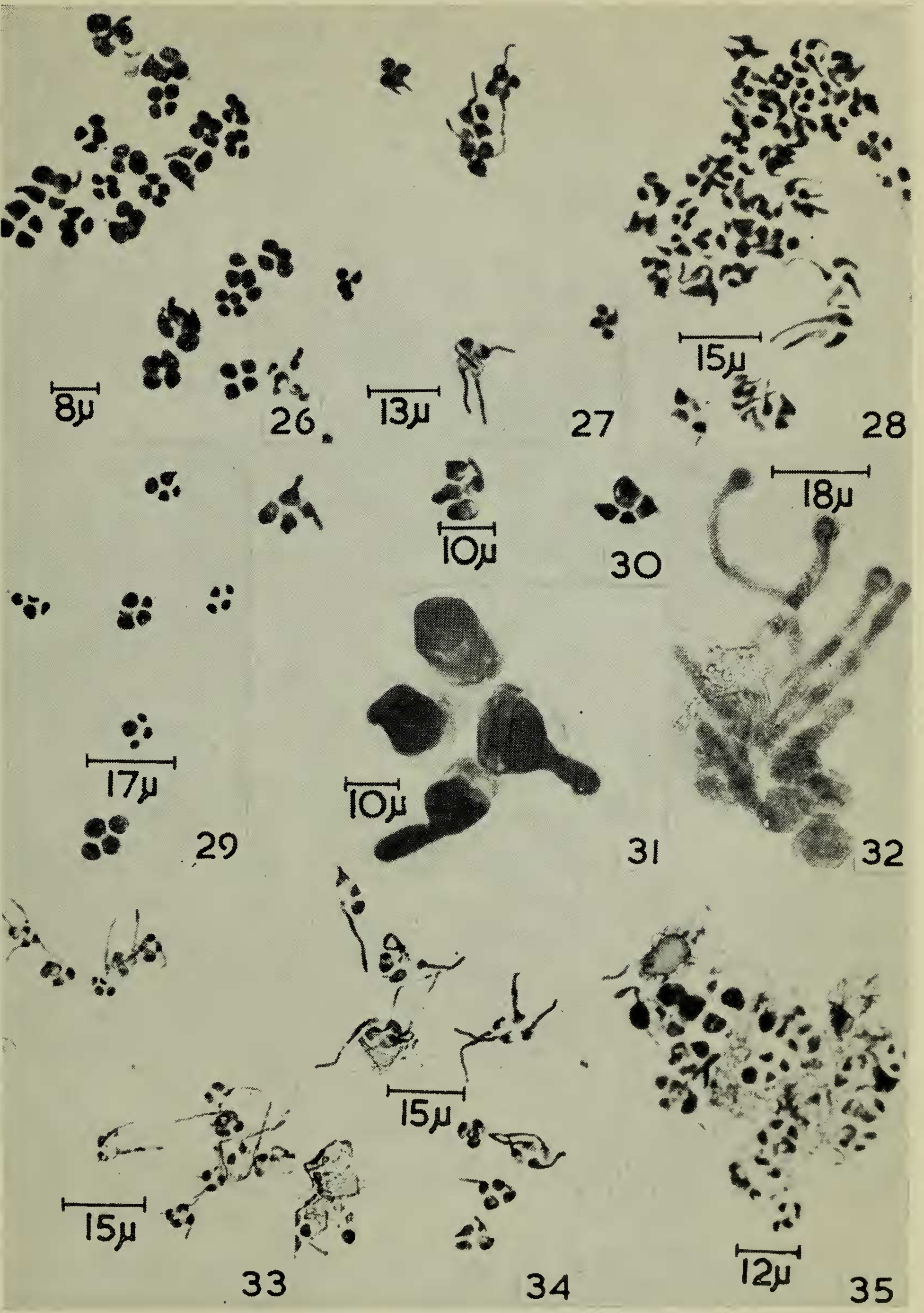
Certain variations were noticed. In *V. Peopoe* a single case of branching was observed in NAA medium ($10^{-4}$ mg/L) and slight swellings of pollen tubes were common in NAA and 2, 4-D media ($10^{-6}$ mg/L). Tips of pollen tubes were pointed in sucrose medium. In *V. Miss Joaquim* oil globules were present in the grains and pollen tubes. Tips or middle portion of pollen tubes were swollen in salt-boron and IAA media (Fig. 22a, 32) and forking of the tube was seen in kinetin medium ($10^{-6}$ mg/L). Exudates were common in GA and K media (Figs. 23, 25).

## Discussion

Structural variation of pollinia in different orchids has been described and the present study reveals the fact that hybrids retain the same genetic characters of parents (Adams, 1959). Further study of other hybrids may substantiate this fact since very few hybrids are critically examined from this point of view. Occurrence of pollen tetrads is common in Orchidaceae (Withner, 1959). The presence of supernumerary spores, frequency of their occurrence, and an analysis of their structure has been studied in the diploid and tetradploids of *Vanda* hybrids (Kamemoto, 1956). In five of the nine hybrids studied presently the supernumerary spores were present and it is interesting to record that only few of them germinated when compared with the regular tetrads.

Of the nine hybrids investigated germination was seen only in *Vanda Poepoe, V. Miss Joaquim* and to a certain extent in *Arachnis Maggie Oei* var. *Yellow Ribbon.* Others did not germinate in the different media used. This loss of germinability may result from immaturity of pollen, long preservation, or it can be an inherent property resulting from complicated and forced hybridisation (Miwa, 1937). It is possible that the reason mentioned last is applicable here since fresh pollinia were used in all the experiments. Except *Vanda,* most of the other hybrids studied presently do not set fruits either by self or cross pollination.

In control medium the percentage germination was low and tube lengths shorter in both the hybrids of *Vanda.* In those cases where the pollen grains germinate with short tubes, it is said that the grains contain a certain amount of nutrients that promote initial growth but this is insufficient for the growth of longer tubes (Brink, 1924a). All the growth adjuncts used presently, both organic



Figures 26–35: *V. Poepoe.* Fig. 26: Germination in dist. water (control). Fig. 27: in stigmatic extract (2 stigmas/10 ml of dist. water). Fig. 28: in 6% sucrose medium, note beak like tubes. Figs. 29-35: *V. Miss Joaquin.* Fig. 29: Tetrads of different sizes. Figs. 30 & 31: Germination in dist. water (control) and one of the tetrads enlarged to show short tubes in Fig. 31. Fig. 32: Germination in IAA medium ($10^{-5}$mg/L), showing pollen tubes with bulbous tips. Fig. 33: in stigmatic extract (2 stigmas/10 ml dist. water). Fig. 34: in borax medium ($10^{-1}$ mg/L). Fig. 35: Aborted grains of different sizes.

and inorganic, were found to be stimulatory when compared with the control medium. Their effects on percentage germination and tube growth were of different magnitude. Better results were obtained for *Vanda* hybrids in stigmatic extract medium which indicated that the necessary stimulii required for germination and tube growth were present. The stimulatory effect of stigmatic extract on pollen germination was observed in other orchids studied earlier (Curtis and Duncan, 1947; Rao and Lee, 1972). Details pertaining to other angiosperms are also reviewed (Johri and Vasil, 1961).

Sucrose undoubtedly had a stimulatory effect on germination and tube growth of *Vanda hybrids*. The optimal concentration varied from 4–6% and best results were obtained in sucrose media. This is in conformity with earlier findings of other workers (Miwa, 1937; Curtis and Duncan, 1947). Externally supplied sugars do serve as a source of nutrition for pollen germination in most of the species studied so far and the important role of sugars is vividly discussed (Johri and Vasil, 1961; O'Kelly, 1955, 1957; Visser, 1955).

In case of *V. Miss Joaquim*, very good germination and tube growth resulted in boron medium. *Vanda Poepoe* showed an improvement both in germination and tube growth but the effect was not as good as stigmatic extract or sucrose media. *Arachnis* Maggie Oei var. (*Yellow*) *Ribbon* also showed good germination in the presence of low concentration of borax but the tube growth was poor.

Salt-boron medium was more effective than borax medium in *V. Poepoe* and it was contrary in case of *V. Miss Joaquim*. In case of the latter the presence of salts reduced both the percentage germination and tube length, though boron was present as part of the medium. In case of *V. Poepoe* borax alone was not stimulatory but its synergistic action with the salts was obvious. Certain salts, especially calcium (ions), have a stimulatory effect on pollen germination in majority of angiosperms so far investigated (Brewbaker and Kwack, 1964; Mascarenhas and Machlis, 1964; Vasil, 1960, 1964). Negative chemotropic response to calcium ions has also been observed in *Lilium, Zea* and others (Rosen, 1968, Cook and Walden, 1965). The results obtained in *Vanda Miss Joaquim* support the above observations.

The fact that pollen grains are naturally endowed with auxins is wellknown and in certain cases like *Antirrhinum, Bryophyllum* the auxin concentration is so high that it is inhibitory to germination (Curtis and Duncan, 1947; Smith, 1942; Vasil, 1960). In the two *Vanda* hybrids studied, all the auxins (concentration $10^{-7}$ to $10^{-4}$ mg/L) had a stimulatory effect on germination and tube growth. The results obtained presently are similar to the observations made in other angiosperms like *Cucumis melo, Eriobotrya japonica, Triticum* and others (Loo and Hwang, 1944; Dikshit, 1956; Vasil, 1960). Curtis and Duncan (1947) found that NAA did not increase pollen germination in the three orchids namely, *Cyrtopodium punctatum, Cattleya guatmalense,* and *Phalaenopsis* hybrid. However, this could be attributed to the comparatively high concentrations used (0.1 – 10 mg/L) when compared with present studies.

Though the effect of gibberellic acid and other gibberellins on germination and elongation of pollen tubes has been extensively studied in other angiosperms, they have not been used before for orchid pollen (Chandler, 1957; Kato, 1955). In both the hybrids of *Vanda* the percentage germination and tube growth improved in low concentration of GA media. Johri and Vasil (1961) postulated that incorporation of kinetin in nutrient medium together with auxins may hasten the division of generative cell. Kinetin improved the percentage germination as well as tube growth of *Vanda Miss* Joaquim and the results were much better than in control, salt-boron, or certain auxin media and similar results were recorded in